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Cell Culture Insert

The invention relates to a cell culture insert comprising a beaker-shaped insert wall having a membrane filter bottom and projecting support arms that are distributed around the circumference of the top and having lateral spacers for a vertical and horizontal orientation in a well with a liquid culture medium in a cell culture plate.

Cell culture inserts, in order to be easy to work with, should have a large feed opening. Also, the lower cross section with the membrane should be sufficiently large to permit a good fluid exchange between the beaker content and well content. It is also advantageous to suspend the cell culture inserts in the well in order not to have any feet at the lower edge of the insert that interfere with the cell growth. In addition to the accessibility of the cell culture insert through the feed opening, the accessibility of the well bottom for pipets is also important.

Cell culture inserts in beaker shape are known, from patent document US 4,871,674 and US 5,578,492 for example, which are suspended in a well and permit access to the cell culture insert and to the well. The cell inserts that are described here are constructed symmetrically and, in their suspended condition, have only small feed windows around the inserts for inserting pipets into the well.

Patent document US 4,871,674 describes that the cell culture insert can be moved into an upper position to enlarge the feed window for inserting a pipet. In the process, the cell culture insert rests against the wall of the well, resulting in the cells in the well being damaged.

Furthermore, from US 5,272,083, a cell culture insert is known that carries on an upper flange a support arm arrangement, which conically narrows by less than 45 degrees until it reaches a cylindrical culture chamber, with cutouts being provided between the support arms that provide pipet accessibility. The culture chamber in this case is significantly constricted, to approximately ¼ of the diameter of the receiving space.

The known cell culture inserts are produced either of a completely colorless transparent material or of the same single-color plastic as the cell culture plate with the wells. This makes it difficult to differentiate between the well and the cell culture insert, thus rendering manual or automated work more difficult.

It is the object of the invention to provide an improved cell culture insert with a sufficiently large membrane surface in which the feed window for the well is significantly larger and in which no moving of the insert is required to insert pipets into the feed window.

This object is met in such a way that the spacers around the circumference of the cell culture insert are distributed in such a way, and implemented with different lengths to the side in such a way, that one large feed window and multiple smaller windows are created.

Advantageous embodiments of the invention are specified in the subclaims.

The beaker-shaped cell culture insert hangs from support arms on the upper edge of the well and is positioned asymmetrically by means of spacers having different lengths. In the process, a feed window, into which pipets can comfortably be inserted, is created between the longest spacers.

Three spacers, which are distributed around the circumference over more than half the circumference are required as a minimum to position the cell culture insert in the well opening in a defined manner. The largest feed window is created if one of the three spacers is shorter than the other two.

This arrangement has the advantage that the shortest spacer determines a minimum space between the cell culture insert and the wall of the well in such a way that capillary narrow spaces of cells between the cell culture insert and the wall of the well are prevented, so that no liquid rises there.

Advantageously, one short spacer and two spacing webs of equal length are arranged equally spaced around the circumference of the beaker. The spacing webs which, when the cell culture insert is inserted into the well, perform an asymmetrical positioning therein, are advantageously downwardly tapered. The support arms ensure that the cell culture insert is suspended in the well at a defined elevation. The shortest spacer ensures that a minimum space is maintained between the walls of the cell culture insert and the well.

In a particularly advantageous embodiment at least one wall cutout, which is provided in the insert wall at its upper end between the long spacers, creates further improved accessibility for pipets. The depth of the cutout is approximately 20% of the height of the insert. The lower edge of the wall cutout has an adequate safe distance to the liquid culture medium. To prevent critical bending stresses, the cutout edge extends upwardly diverging and rounded toward the support arms. The insert wall has a relatively large diameter in the culture chamber since it is provided only with shaping inclines of approximately 1.5 degrees on the outside and 3.3 degrees on the inside. As a result, the free membrane diameter is larger than the radius of the well. If wall

cutouts are provided between all support arms, not only the pipet accessibility is significantly enhanced, but also the tweezer access to the support arms.

Despite the eccentric suspension of the cell culture insert in the well, a sufficiently large observation window of, e.g., 3 mm diameter remains over the center of the well for the automated optical evaluation of the cell culture plates with the above described cell culture inserts.

If a larger feed window is required, the cell culture insert can be pushed up with a pipet in such a way that it can slide a short distance out of the well along the inclined spacers. After removing the pipet, the cell culture insert reliably slides back into the well along the spacers.
The high enclosed construction of the cell culture insert prevents a contamination between the inside and outside of the cell culture insert.

For handling the cell culture insert in the well, it can easily be grasped on one of the long support arms, inserted, and also lifted back out, with the aid of a pair of tweezers.

Any known membrane for cell culture inserts may be used; capillary membranes of polyester or polycarbonate have the advantage of a precisely adjustable porosity and transparency.

The cell culture insert is advantageously produced of a tinted plastic that represents a visible contrast to the material of the well. This improves the recognizability of the feed opening. Preferably a transparent colored plastic will be used.

An embodiment of the invention is described in the figures by way of example.

Fig. 1 shows a top view of a cell culture insert of a first embodiment in the well,

Fig. 2 shows a view of the cell culture insert from the side with three spacing webs,

Fig. 3 shows a view of the cell culture insert from the side with spacing webs and spacer,

Fig. 4 shows a sectional view of the well with cell culture insert with spacer,

Fig. 5 shows a sectional view of the well with cell culture insert with inclined wall,

Fig. 6 shows a second embodiment of the insert in the perspective;

Fig. 7 shows an axial section A-A for the above,

Fig. 8 shows a top view for the above.

Fig. 1 shows a cell culture insert 1 of a first embodiment in a top view, showing how in a round opening in the well 2, a cell culture insert 1 is held eccentric to the opening. It is held by three support arms 3, 4, wherein the support arm 3 is implemented shorter than the other two. This creates a large feed window 5, into which a pipet can easily be inserted, as well as two smaller windows 5A. The opening 6 of the cell culture insert is also easily accessible from above. The support arms 3, 4, each are arranged offset by 120 degrees. The eccentricity between the axis of the well and that of the insert is more than 1.3 mm, e.g., 4 mm. It is achieved by means of different spacing webs that are arranged below the support arms 3, 4.

Fig. 2 shows a side view of the beaker-shaped cell culture insert 1. On it, long triangular spacing webs 7 are affixed laterally, tapering downward, and another shorter spacing web 7a. At the top, the support arms 3, 4, are formed on the insert wall 11, of which the support arm 3 is shorter, which belongs to the shorter spacing web 7a.

Fig. 3 shows a side view of the beaker-shaped cell culture insert 1 in a modified design. On it, the triangular spacing webs 7 and another spacer 8 in knob-shape are affixed.

Fig. 4 shows a section through the cylindrical well 2 with suspended cell culture insert 1. The support arms 3, 4 rest on the top of the well 2 and determine the elevation of the cell culture insert 1. Via the spacing webs 7 and the spacer 8, the space between the walls is maintained in a defined manner. The feed window 5 is located between the long support arms 4. The cell culture insert 1 has at its top the opening 6 and at its bottom the membrane 9. The space between the walls at the level P of the fluid surface 10 is sufficiently large so that no capillary effect occurs.

Fig. 5 shows the same section as Fig. 4, with the difference that the space between the walls of the insert 1 and well 2 at the height of the fluid surface 10 is determined by the relatively greater incline of the cell culture insert 1, which is approximately 7 degrees in the example.

Fig. 6 shows, in the perspective, an additional embodiment of a cell culture insert 1A, wherein the insert wall 11 has a wall cutout 12 from the top between the support arms 3, 4 in each case that is designed rounded toward the support arms. The cutout depth T is approximately 20% of the insert height H, so that the lower edge 13 of the wall cutouts 12 has a sufficient level distance H to the normal fluid surface 10.

Fig. 7 shows an axial cross section through the embodiment in Fig. 6. In the figure, the size ratio of the cutout depth T to the insert height H is apparent, the latter being approximately five times larger. The normal level P of the liquid culture medium 10 is also shown, which is located approximately 1/3 of the insert height H above the membrane 9. The insert wall 11 is conically narrowed on its outside with a shaping incline of approximately 1.5 degrees and tapered on its inside with approximately 3.3 degrees so that the upper wall thickness WO increases toward the bottom from 1 mm to a lower wall thickness WU of 1.5 mm, thus creating a secure sealing edge

at the bottom for the membrane 9 and more circumferential free space at the top.

Fig. 8 shows a top view of Fig. 7. The interior wall W of the well has been marked with a dot-and-dash line, the well radius R of which is smaller than the membrane diameter D. The eccentricity of the insert 1a to the interior well wall W is approximately 1.5 mm in the example. The well radius R is 10 - 12 mm, for example, and the usable diameter D of the membrane 9 is 11- 13 mm. The insert wall 11 is approximately 15 - 17 mm wide at the top. The spacers 7, 8, through their different lateral extension, create an eccentricity of the insert 1A in the well 2 of 1.3 - 1.7 mm.

List of Reference Numerals

1	1 A Cell culture insert	P	Level
2	Well	R	Well radius
3	Short support arm	T	Cutout depth
4	Long support arm	W	Interior well wall
5	Feed window, large	WO	Upper wall thickness
5a	Feed window, small	WU	Lower wall thickness
6	Opening of the cell culture insert		
7	Long spacing web		
7a	Short spacing web		
8	Spacer		
9	Membrane		
10	Fluid surface		
11	Insert wall		
12	Wall cutout		
13	Lower edge of the wall cutout		
A	Level distance to the lower edge		
D	Membrane diameter		
H	Insert height		
N	Liquid culture medium		